



Increased expression of Interleukin-13 but not Interleukin-4 in cystic fibrosis patients

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Abstract

Background: Many patients with cystic fibrosis (CF) suffer from allergic disease, which can complicate treatment of CF lung disease. Interleukin (IL)-4 and IL-13 have been shown to be important mediators in allergic disease. **Objective:** To investigate the role of IL-4 and IL-13 in allergic and non-allergic CF patients. **Methods:** Expression of IL-4 and IL-13 mRNA was investigated in peripheral blood mononuclear cells (PBM) of seven CF patients with allergy, of six patients without allergy and of nine healthy subjects as well as in BAL cells of four patients and of all controls. PBM from six patients were incubated with recombinant human IL-13 or human antiIL-13 antibody without and with LPS stimulation and TNF α levels were measured by ELISA. **Results:** IL-13 mRNA expression was increased in allergic and non-allergic patients compared to controls. No significant difference in IL-4 expression could be found between patients and controls. Addition of IL-13 decreased TNF α in PBM culture supernatants. **Conclusion:** Our data suggest that IL-13 rather than IL-4 might play an important role in both allergic and non-allergic CF patients. IL-13 might also compromise host defence by decreasing TNF α production.

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1. Introduction

Cystic fibrosis (CF) is the most common life shortening autosomal recessive disorder in Caucasian people [1]. The main cause for morbidity and mortality is the involvement of the lung. Chronic infection and inflammation lead to bronchiectasis and obstructive pulmonary disease. Many patients also develop allergic diseases like bronchial asthma and allergic bronchopulmonary aspergillosis (ABPA): sparsely the mold *Aspergillus fumigatus* accelerates pulmonary deterioration [2,3]. Moreover, approximately 50% of all CF patients have elevated levels of immunoglobulin E (IgE) in serum [2]. Allergic disease in CF patients can complicate both

diagnosis and treatment of infectious exacerbations. However, the underlying pathomechanisms are far from being completely understood.

Interleukin-4 (IL-4) and Interleukin-13 (IL-13) are two cytokines which have been shown to be involved into pathophysiological reactions in allergic disease by regulating IgE production by B cells [4,5]. IL-4 is produced by Th2 lymphocytes which are implicated in numerous pathophysiological reactions related to allergen responses and eosinophilia [6]. In CF, IL-4 was described to be involved into the pathogenesis of ABPA [7,8]. IL-13, a more recently described cytokine is sharing some biological properties with IL-4 [9].

We hypothesised that IL-4 and IL-13 might play an important role in the pathophysiology of allergic reactions in CF patients. Therefore, we sought to investigate IL-4 and IL-13 expression in allergic and in non-allergic CF patients.

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2. Patients and methods

Thirteen adult CF patients were investigated (10 patients were homozygous for $\Delta F508$, no genotyping was available for the other 3). This group was divided into two subgroups: allergic patients ($n=7$) and non-allergic patients ($n=6$). Allergy was defined by elevated serum IgE levels (>100 kU/l), >1 positive skin test and/or elevated IgE in RAST and a history of allergy. *Pseudomonas aeruginosa* was found in sputum culture of all patients. Three patients received systemic corticosteroids (2 allergic, 1 non-allergic). 9 healthy volunteers served as control group.

Pulmonary function was measured in all patients but one and in all control subjects by spirometry, bodyplethysmography (BodyScope, Medizin Electronic GmbH, Germany) and gas check from capillary blood (Compact 2 Blood Gas Analyzer, AVL, Austria). The forced expiratory volume in 1 s (FEV1) and the vital capacity (VC) were selected to describe the status of the lung.

Blood samples of all patients and controls were obtained for peripheral blood mononuclear cells (PBM). Total immunoglobulin E (IgE) and specific IgE to common allergens was determined using the Unicap 100 system (Pharmacia, Uppsala, Sweden).

After informed consent was obtained four patients and all control subjects underwent fiberoptic bronchoscopy. Bronchoalveolar lavage was performed by instilling 160 ml of sterile 0.9% saline solution in 20 ml-aliquots into the middle lobe and by withdrawing the fluid immediately.

2.1. Cell isolation and RNA preparation

Peripheral blood mononuclear cells (PBM) were isolated by centrifugation over Ficoll-Paque (Pharmacia, Uppsala, Sweden) and washed twice with PBS containing 2% fetal calf serum (Seromed, Berlin, Germany). Cells of the bronchoalveolar lavage (BAL) were isolated by centrifugation and washed once with PBS containing 2% fetal calf serum. RNazol (Wak, Bad Soden, Germany) was added and samples were stored at -20°C until further preparation. RNA was extracted by treatment with chloroforme and precipitated with isopropanol.

2.2. RT-PCR

RNA was reverse-transcribed using 2 μl 25 mM MgCl_2 , 1 μl 10 \times PCR Buffer, 1 μl 10 mM of each deoxynucleotide triphosphate (dNTP), 0.5 μl 50 μM OligodT, 0.5 μl 20 U/ μl RNase inhibitor and 0.5 μl 50 U/ μl reverse transcriptase (Perkin Elmer Biosystems, Roche, Branchburg, USA). The mixture was incubated at 42°C for 30 min and at 99°C for 5 min. Samples were stored at -20°C until amplification.

The resultant cDNA was amplified by PCR in a thermal cycler (Hybaid, Teddington, UK) with a final volume of 100 μl containing 10 μl cDNA (IL-13, IL-4) or 5 μl cDNA (β -actin), 10 μl or 15 μl dilution buffer, 8 μl 12 mM MgCl_2 , 8 μl 10 \times PCR buffer, 55.5 μl H_2O with DEPC, 0.5 μl 5 U/ μl recombinant *Taq* DNA polymerase (Perkin Elmer Biosystems, Roche, Branchburg, USA) and 2.0 μl 15 mmol of each primer. The oligonucleotide primers for PCR were based on published mRNA sequences. The β -actin served as control. The human β -actin primers were 5'-GTG GGG CGC CCC AGG CAC CA-3' for the upstream primer and 5'-CTC CTT AAT GTC ACG CAC GAT TTC-3' for the downstream primer; IL-13 utilized 5'-GAG TGT GTT TGT CAC CGT TG-3' for the upstream primer and 5'-TAC TCG TTG GTC GAG AGC TG-3' for the downstream primer. IL-4 primers were 5'-GGA CAC AAG TGC GAT ATC ACC-3' for the upstream primer and 5'-ATT TCT CTC TCA TGA TCG TC-3' for the downstream primer. PCR amplification for β -actin was performed for 36 cycles (1 min at 94°C , 1 min at 60°C , 40 s at 72°C), for 35 cycles (45 s at 95°C , 45 s at 60°C , 90 s at 72°C) for IL-13 and for 38 cycles (1 min at 95°C , 1 min at 60°C , 1 min at 72°C) for IL-4.

2.3. Identification of PCR products

PCR products were analysed by electrophoresis on a 2% agarose gel containing ethidium bromide and visualized by UV light. The sizes of the PCR products were compared with the expected PCR product length and molecular weight markers (Boehringer, Mannheim, Germany).

2.4. Recombinant human Interleukin-13 and anti-Interleukin-13

Recombinant human IL-13 (rhIL-13) was used at a concentration of 5 ng/ml as indicated by the manufacturer (R and D Systems, Minneapolis, USA). Monoclonal anti-human IL-13 (antiIL-13) concentration was 2 $\mu\text{g}/\text{ml}$ to inhibit IL-13 activity as indicated by the manufacturer (R and D Systems, Minneapolis, USA).

2.5. Tumor necrosis factor α enzyme-linked immunosorbent assay (ELISA)

A tumor necrosis factor α (TNF α) ELISA (R and D Systems, Minneapolis, USA) was used to determine the concentration of TNF α in cell culture supernatants of PBM after 18 h of incubation in a group of six patients with and without stimulation by 0.2 mg/ml lipopolysaccharide (LPS) (for the whole time of incubation) and addition of rhIL-13 or antiIL-13. TNF α concentrations were determined by comparison with recombinant stan-

Table 1
Characteristics of patients and controls

	Allergic CF	Non-allergic CF	Controls	<i>P</i> ^c
Number	7	6	9	
Median age (years)	25.0	28.5	27.4	
Males/females	3/4	3/3	4/5	
IgE (kU/l)*	400.5 ± 23.9	61.2 ± 21.4	30.8 ± 12.6	<0.05/ <0.05/n.s.
FEV1 (%)*	42.3 ± 5.5	46.5 ± 7.9	98.6 ± 1.6	n.s./ <0.05/ <0.05
VC (%)*	52.4 ± 5.9	53.6 ± 7.4	96.9 ± 1.8	n.s./ <0.05/ <0.05

*Mean ± S.E.M. ^c*P* values of allergic vs. non-allergic/allergic vs. control/non-allergic vs. control. n.s.: not significant.

dards run parallel with each batch of assay. Minimum detectable dose of TNFα was less than 4.4 pg/ml as indicated by the manufacturer.

2.6. PBM cell culture

PBM were cultured in RPMI medium (Biochrom KG, Berlin, Germany) containing 10% FCS, 1% L-glutamine and 2% penicilline/streptomycine. Temperature was 37.0 °C. Atmosphere contained 5.0% CO₂. Incubation time was 18 h. Cell culture supernatants for ELISA measurements were obtained by centrifugation (3.000 rev./min, 10 min).

2.7. Statistics

Mean values of the data were compared by Mann–Whitney test. A *P* value <0.05 was considered significant.

3. Results

3.1. IgE serum protein levels, FEV1 and VC

IgE serum protein, FEV1 and VC mean values are shown in Table 1. IgE serum levels were significantly increased in allergic patients in comparison to non-allergic patients and controls (*P*<0.05) whereas IgE serum protein levels were not statistically different between non-allergic patients and controls.

3.2. PBM and BAL cell counts

There was no significant difference in PBM total as well as differential cell counts between both patient groups. PBM and BAL cell counts for patients and controls are shown in Table 2.

3.3. Expression of IL-13 mRNA

PBM of all patients showed IL-13 expression. In contrast, IL-13 mRNA was virtually absent in PBM of normal control subjects. IL-13 mRNA expression could be detected in all of the investigated samples of BAL cells in CF patients (Fig. 1). In control subjects, IL-13 expression could only be detected in two BAL samples in very low amounts.

3.4. Expression of IL-4 mRNA

In PBM IL-4 mRNA was expressed in all patients and all but one of the tested control persons. IL-4 mRNA expression could neither be detected in BAL cells of CF patients nor in the healthy control subjects (Fig. 2).

3.5. Effect of stimulation of PBM with rhIL-13 on TNFα production

Protein concentrations of TNFα in cell culture supernatants of PBM after 18 h of incubation were determined

Table 2
PBM and BAL cell counts

	Allergic CF patients	Non-allergic CF patients	Controls
PBM total (×10 ⁶ /ml)	13.9 ± 2.4	10.8 ± 2.1	9.8 ± 1.6
Monocytes (%)	37.9 ± 7.5	36.8 ± 6.4	32.4 ± 1.1
Lymphocytes (%)	27.4 ± 5.9	26.7 ± 6.2	41.7 ± 3.6
Neutrophils (%)	34.5 ± 9.2	36.3 ± 6.6	25.8 ± 4.4
Eosinophils (%)	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
BAL total (×10 ⁶ /ml)	23.4 ± 13.3	45.4*	3.9 ± 0.6
Alveolar macrophages (%)	6.8 ± 3.2	9*	84.5 ± 2.1
Lymphocytes (%)	1.7 ± 0.4	1*	10.2 ± 1.8
Neutrophils (%)	91.3 ± 3.7	90*	5.2 ± 1.6
Eosinophils (%)	0.2 ± 0.2	0*	0.1 ± 0.1

Mean ± S.E.M. *: Only one BAL sample could be investigated.

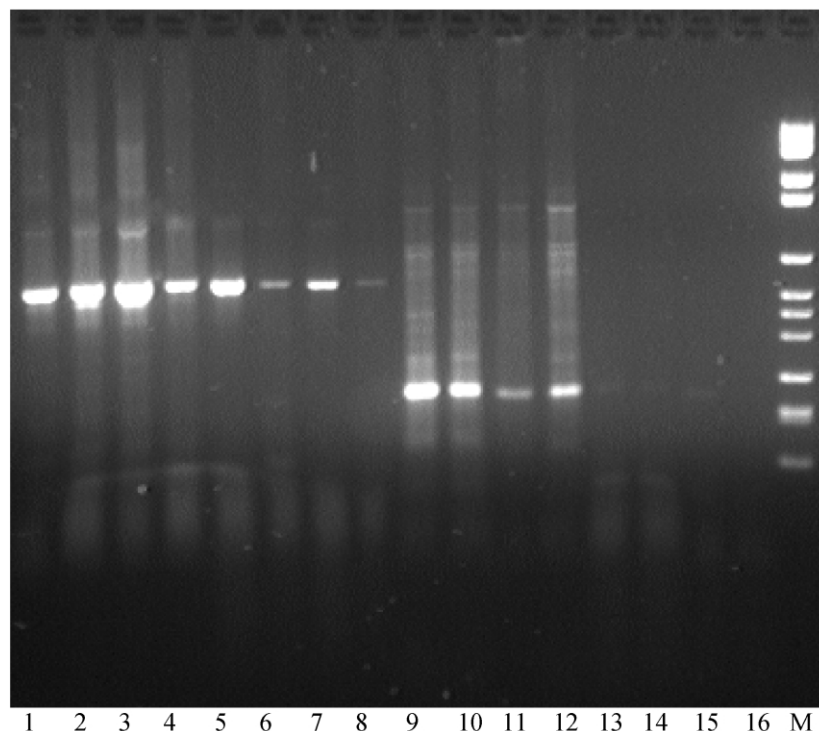


Fig. 1. RT-PCR of IL-13 mRNA expression in BAL cells and PBM of patients and controls. Lanes 1–8: β -actin in BAL cells (1,2) and PBM (3,4) of two patients and in BAL cells (5,6) and PBM (7,8) of two control persons as internal standard. Lanes 9–16: IL-13 is expressed in BAL cells (9,10) and PBM (11,12) of the patients but only in low amounts in BAL cells (13,14) and PBM (15,16) of the control persons. Length of IL-13 transcript was 275 bp as expected. M: molecular weight marker.

by ELISA in samples of six patients. RhIL-13 and antiIL-13 were utilised with and without LPS stimulation (Fig. 3). $\text{TNF}\alpha$ protein concentrations were 20.69 ± 11.76 pg/ml after 18 h of incubation and increased to 760.82 ± 471.40 pg/ml after stimulation with LPS. RhIL-13 decreased $\text{TNF}\alpha$ levels to 16.25 ± 6.11 pg/ml without ($P > 0.05$; not significant) and 259.07 ± 219.79 pg/ml after stimulation ($P < 0.05$). After adding antiIL-13, $\text{TNF}\alpha$ concentrations were increased without (43.69 ± 26.96 pg/ml; $P < 0.05$) and with stimulation by LPS (818.15 ± 445.73 pg/ml; not significant) (Fig. 3).

4. Discussion

In the present study increased IL-13 mRNA expression in PBM of CF patients could be demonstrated in both allergic and non-allergic patients in comparison to healthy non-allergic subjects. Moreover, analysis of BAL cells revealed increased IL-13 expression in allergic and non-allergic patients and only small expression in the control group. However, there was no significant difference in IL-4 expression in PBM between patient groups and the control group. No IL-4 mRNA expression was found in BAL cells of patients or controls.

These findings indicate that IL-13 might play a more important role in CF than IL-4. Moser et al. reported a

Th2 predominated immune response in CF patients chronically infected with *Pseudomonas aeruginosa* [10]. In that study increased secretion of IL-4 from PBM after stimulation was found. Although we found *Pseudomonas aeruginosa* in sputum cultures of all patients we did not observe increased IL-4 expression compared to control subjects. However, we investigated non-stimulated cells.

In our study, there was a significant difference in IL-13 expression between allergic and non-allergic patients compared to control subjects. This suggests that IL-13 might be involved in both allergic and non-allergic processes in CF. However, from these data it is not clear whether IL-13 might be more important in mediating allergic reactions in CF than being a typical mediator in chronic *Pseudomonas aeruginosa* infection.

IL-13 might alter directly or indirectly (via induction of other mediators) the lung function in CF patients. It might be also responsible for bronchial hyperresponsiveness, which is a common symptom in CF patients. Since IL-13 expression could be demonstrated in BAL cells it might also act as a local mediator similar to its role in asthma. However, the number of patients in this study was too small to draw any conclusions.

PBM of CF patients may be a major source of IL-13 production in peripheral blood. IL-13 is known to be mainly produced by CD4⁺ T helper cells [11] and

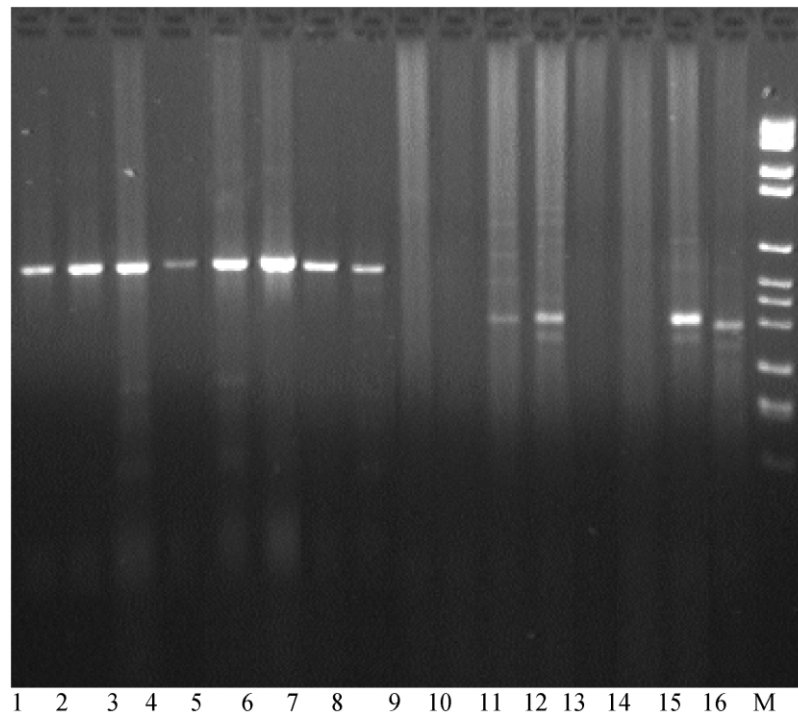


Fig. 2. RT-PCR of IL-4 mRNA expression in BAL cells and PBM of patients and control persons. Lanes 1–8: β -actin in BAL cells (1,2) and PBM (3,4) of two patients and in BAL cells (5,6) and PBM (7,8) of two control persons as internal standard. Lanes 9–16: IL-4 is expressed in PBM of the patients (11,12) and the control persons (15,16) but not in BAL cells of the patients (9,10) or the control persons (13,14). Length of IL-4 transcript was 399 bp as expected. M: molecular weight marker.

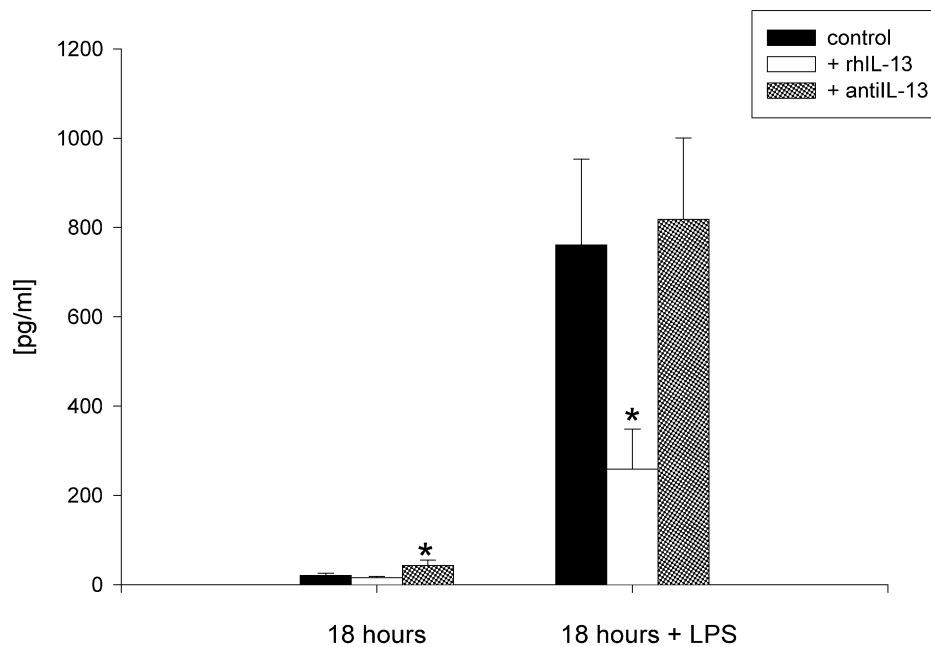


Fig. 3. TNF α protein concentrations in PBM cell culture supernatants. Columns indicate mean values after 18 h of incubation (20.69 pg/ml, control) with rhIL-13 (16.25 pg/ml) and antiIL-13 (43.69 pg/ml) and after stimulation using LPS (760.82 pg/ml, control) with rhIL-13 (259.07 pg/ml) and antiIL-13 (818.15 pg/ml). *: $P < 0.05$ vs. control.

patients had considerable proportions of lymphocytes (27.0 and 26.2%) in PBM.

IL-13 has been reported to have powerful antiinflammatory activities [12]. This might be important in CF in terms of decreasing the host defence against bacterial infections e.g. *Pseudomonas aeruginosa*. Therefore, we investigated the effect of IL-13 on PBM of CF patients. RhIL-13 and antiIL-13 were applied on unstimulated and stimulated PBM and TNF α production was measured. RhIL-13 administration decreased TNF α production in both stimulated and unstimulated PBM. After blocking the effect of IL-13 by using antiIL-13, TNF α production was increased. These data suggest that IL-13 might be involved in regulation of TNF α production in PBM of CF patients. Our data correspond to the results of Cosentino et al. who described a reduction of TNF α secretion of monocytes after IL-13 stimulation [13]. IL-13 might decrease TNF α secretion from PBM in CF thus worsening host defence in case of bacterial infection. The increase of TNF α protein in supernatants in the presence of antiIL-13 antibodies in comparison to without further supports the notion that IL-13 is endogenously expressed and secreted by PBM in CF.

5. Conclusion

IL-13 but not IL-4 expression was increased in CF patients with and without allergy. IL-13 could also be shown to decrease TNF α production from PBM. These results suggest that IL-13 might have antiinflammatory effects in CF patients thus compromising host's defence. Further studies are warranted to study the effects of IL-13 on the lung in CF patients. IL-13 might also be an interesting candidate for a mediator of bronchial hyperresponsiveness in CF.

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